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Comparison of Eleven Commercially Available Kits for the Radioimmunoassay of Serum Thyroxine[1])

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Summary: Eleven of the thyroxine RIA kits commercially available in the Netherlands were compared with respect to reliability, sensitivity, simplicity and performance. The intra- and interassay coefficients of variation ranged from 4.6 to 11.3%, whereas the recovery of added thyroxine ranged from 85 to 114%. The labour-time per 100 tubes varied from 115 to 175 minutes.

Vergleich von elf kommerziell erhältlichen Bestecks für den Radioimmunassay von Thyroxin im Serum

Zusammenfassung: Elf der in den Niederlanden kommerziell erhältlichen Testbestecks für den Radioimmunassay von Thyroxin wurden hinsichtlich Zuverlässigkeit, Empfindlichkeit und Einfachheit der Ausführung verglichen. Die Variationskoeffizienten in der Serie und von Tag zu Tag waren im Bereich von 4,6–11,3% und die Wiederfindung von zugesetztem Thyroxin betrug 85–114%. Hundert Ansätze können in 2–3 Stunden bearbeitet werden.

Introduction

One of the parameters for the evaluation of thyroid function is the serum thyroxine (T_4) level. During recent years many manufacturers have introduced kits for the radioimmunoassay of serum T_4. This study was undertaken to make an inventory of a number of objective criteria with the aim of facilitating the choice of kit.

Materials and Methods

Reagents

The following kits were used and further referred to as mentioned in parentheses:

1. T_4-RIA-PEG; Abbott Laboratories, North Chicago, Ill. U.S.A. (Abbott),
2. T_4 Solid Phase RIA; Becton Dickinson Immunodiagnostics, Orangeburg, NY, U.S.A. (Becton D),
3. Riamat T_4; Byk Mallinckrodt, Dietzenbach-Steinberg, Germany (Byk Riamat),
4. SPAC T_4; Byk Mallinckrodt, Dietzenbach-Steinberg, Germany (Byk Spac T_4),
5. Gamma Coat T_4; Clinical Assays, Cambridge, Ma. U.S.A. (Clinical Assays),
6. Immophase FT_4-RIA[2]); Corning Medical, Midfield, Ma, U.S.A. (Corning),
7. T_4-RIA-Premix; Diagnostic Products Corporation, Los Angeles, Calif., U.S.A. (DPC-Premix),
8. T_4-RIA-Autopak; Micromedic Systems, Horsham, Pa, U.S.A. (Micromedic),
9. Tetratab RIA T_4, Nuclear Medical Laboratories, Dallas, Tx., U.S.A. (NML),
10. T_4-RIA IM 801, The Radiochemical Centre, Amersham, U.K. (RCA IM 801),
11. T_4-RIA PEG IM 921; The Radiochemical Centre, Amersham, U.K. (RCA IM 921).

L-thyroxine (T-2376) was purchased from Sigma (St. Louis, Miss., U.S.A.). All other chemicals were reagent grade mainly from Merck (Darmstadt; Germany).

Sera

Sera from hypothyroid (C2), euthyroid (C4, C5) and hyperthyroid patients (C7 – C10) were obtained from the Central Blood Bank, Amsterdam, the General Zeister Hospital, Zeist and the Antonius Hospital, Utrecht. The sera C3 and C6 were prepared by pooling of sera with various T_4 levels.

Procedure

For the determination of the intra- and interassay variation for each kit four experiments were carried out, two with one kit specimen and two with another, originating from a different batch. In each experiment the nine sera C2–C10, were measured in triplicate. For the determination of the percentage recovery in

[1]) Reference to a company and/or product is only for the purpose of information and identification and does not imply approval or recommendation of the company and/or product by the National Institute of Public Health to the exclusion of others which may also be suitable.

[2]) The Corning Immophase FT_4-RIA, although meant for the determination of free serum T_4 also yields a value for total serum T_4.

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a fifth experiment, 75, 150 and 225 nmol/l T_4 were added to T_4-free serum, which had been prepared as follows:

16 g charcoal was added to 100 ml serum; after mixing for 20 h at 4 °C the suspension was centrifuged for 60 min at 7000 *g*; the serum was decanted and centrifuged for 60 min at 13 000 *g*; finally the serum was filtered through a 0.22 μm millipore filter.

Results

Table 1 shows technical data from each kit, such as the way in which T_4 is liberated from the binding proteins, the number of standards, with matrix, incubation time and incubation temperature, separation method and the total amount of radioactivity contained.

The intra- and interassay coefficients of variation (tab. 2) for the sera C_2–C_{10} were calculated as outlined by *McDonagh* et al. (1).

In table 3 the mean T_4 concentrations of each serum sample as measured by all kits are presented. To determine whether any kit systematically yields high or low T_4 values all kits were ranked from 1 to 11 according to their results for each serum sample. The kit yielding the lowest T_4 value for serum C2 was given number 1, the kit yielding the next higher value was given number 2 etc. The same procedure was followed for the other sera except serum C9, since this serum did not fall within the measuring range for all kits. For each kit the sum of ranking numbers was calculated by adding the individual numbers. According to *Thompson & Willke* (2) the "critical values" for the sum of ranking numbers for 8 sera and 11 kits are 23 and 73 (with 95% confidence level). According to these figures one kit, Byk-Riamat, measures significantly low values, whereas three kits (Abbott, Becton D and NML) measure too high.

The percentage recovery of known amounts of T_4 added to T_4-free serum as measured by each kit is presented in table 4. For the kits with no zero control sample the response of the T_4-free serum was much lower than that of the first standard point. For the kits containing a zero sample there was no significant difference in response

Tab. 1. Technical data of T_4 RIA kits.

Kit	Liberation of T_4 from TBG/ albumin	Standards range: nmol/l no. – matrix	Sample volume (μl)	Incubation time (min)	Incubation temp. (°C)	Separation method	$[^{125}I]T_4$ per 100 tubes (kBq)
Abbott	ANS + Thiomersal Barbital	0 – 309 5 – AS	25	60[1] 120	37 RT	PEG	< 166
Becton D	ANS Barbital	0 – 309 6 – HS	10	45[1] 50	37 RT	ACT	< 333
Byk-Riamat	ANS Barbital	0 – 515 7 – HS	10	30 60[1]	37 RT	ion-exchange resin	< 148
Byk-Spac T_4	ANS Barbital	0 – 515 7 – HS	25	60[1] 120	37 RT	ACT	< 148
Clinical Assays	ANS Na-salicylate	0 – 386 6 – HS	10	45	RT	ACT	< 185
Corning	Thiomersal	13 – 315 5 – HP	25	20 + 30	RT	immuno-sorbent	< 247
DPC-Premix	ANS Barbital	0 – 309 6 – AS	25	20 30[1]	RT 50	2nd antibody body + PEG	< 222
Micromedic	ANS Barbital	0 – 412 7 – HP	20	60	37	ACT	< 370
NML	0.025 mol/l HCl Barbital	15 – 304 5 – HS/AS	10	30–60 60[1]	RT RT	$(NH_4)_2SO_4$ precipitation	< 370
RCA-IM 801	Thiomersal	6.4 – 275 4 – HS	50	60	37	Adsorption powder	< 444
RCA-IM 921	Thiomersal	12 – 275 4 – HS	50	45	RT	PEG	< 444

ANS = 8-anilino-1-naphthalene sulphonic acid;
AS = animal serum;
HS = human serum;
HP = human plasma;
PEG = polyethyleneglycol (M_r = 6000);
ACT = antibody-coated tube;
RT = room temperature.
[1] denotes the conditions chosen here in the case of several prescribed possibilities.

Tab. 2. The intra- and interassay coefficients of variation for 11 thyroxine kits.

Kit/ Serum	C2	C3	C4	C5	C10	C6	C7	C8	C9
					CV[1] intraassay (%)				
Abbott	7.5	4.6	3.2	2.5	2.1	*2.3*	1.1	2.5	4.0
Becton D	*5.2*	3.6	4.6	3.2	5.3	7.5	6.2	5.4	3.1
Byk-Riamat	14.3	8.3	7.9	4.9	9.4	13.6	4.6	*8.2*	5.1
Byk Spac T_4	3.8	3.9	6.4	3.1	4.2	*3.7*	3.3	1.4	3.2
Clinical Assay	10.6	7.2	5.6	3.0	*4.0*	3.3	3.0	4.0	3.2
Corning	3.8	1.8	3.3	3.5	4.1	2.1	2.4	1.5	*2.5*
DPC-Premix	2.9	3.0	*3.1*	3.8	2.9	2.0	3.4	3.1	5.4
Micromedic	6.4	*4.7*	5.4	5.2	4.2	3.5	3.8	4.9	4.2
NML	6.1	3.8	2.4	2.5	2.8	4.7	5.2	*3.0*	3.8
RCA IM 801	2.0	2.9	1.4	1.8	1.5	2.3	2.4	1.7	*1.8*
RCA IM 921	5.2	4.1	2.0	2.6	1.8	*3.0*	4.0	4.0	2.6
					CV[2] interassay (%)				
Abbott	8.9	4.6	7.6	*4.3*	4.8	3.8	2.5	2.9	4.0
Becton D	15.7	13.1	*9.5*	10.3	9.6	8.0	9.3	9.0	5.9
Byk-Riamat	19.2	10.9	7.9	8.1	9.4	13.6	6.6	*9.2*	5.1
Byk Spac T_4	*6.3*	3.9	6.7	7.8	8.3	3.7	6.6	4.5	5.6
Clinical Assays	15.5	9.7	*8.9*	9.1	6.0	7.3	9.6	8.5	7.2
Corning	13.0	3.2	*4.4*	4.5	4.7	2.2	2.4	3.8	7.3
DPC-Premix	5.1	6.5	5.5	5.8	*6.2*	5.6	7.0	11.2	14.1
Micromedic	9.4	*9.1*	10.6	10.6	6.4	6.6	6.2	7.6	13.6
NML	14.4	7.5	7.7	5.7	*7.8*	15.6	9.2	9.8	6.9
RCA IM 801	16.9	9.1	7.3	8.9	*8.5*	9.9	7.9	6.6	8.2
RCA IM 921	12.0	*11.4*	8.1	10.7	7.4	10.1	13.9	16.9	13.3

[1] In four assays the T_4-concentration was measured in triplicate
[2] N = 4
[3] Median values per kit are set in italics

Tab. 3. T_4 concentrations and ranking numbers per kit for the sera C2–C10.
4 assays in triplicate; N = 12.

Kit	C2		C3		C4		C5		C6		C7		C8		C9		C10		Sum of ranking numbers
	nmol/l	no.	nmol/l	no.	nmol/l	no.	nmol/l	no.	nmol/l	no.	nmol/l	no.	nmol/l	no.	nmol/l	no.	nmol/l	no.	
Abbott	41.8	8	62.5	8	117	9.5	135	10	176	9	217	9	264	10	298	–	146	11	74.5
Becton D	43.5	9	65.9	9	118	11	139	11	180	10	220	11	249	6	274	–	138	9	76
Byk-Riamat	28.1	1	47.0	2	76.3	1	96.3	1	133	1	173	1	202	1	216	–	104	1	9
Byk-Spac T_4	34.2	4	56.4	5	100	4	118	4	156	3	184	2	225	4	248	–	124	5	31
Clinical Assays	38.1	7	59.3	6	108	7.5	124	5.5	160	5	205	4.5	233	5	255	–	124	5	45.5
Corning	54.5	11	68.3	10	104	5.5	129	8	164	6	205	4.5	254	9	311	–	139	10	64
DPC-Premix	35.5	5	61.4	7	108	7.5	128	7	167	7	208	6.5	250	7	>309	–	135	7	54
Micromedic	32.6	3	54.8	4	96.3	3	114	3	146	2	186	3	210	2	246	–	116	3	23
NML	50.7	10	72.8	11	117	9.5	132	9	182	11	219	10	269	11	298	–	137	8	79.5
RCA-IM 801	32.5	2	52.6	3	104	5.5	124	5.5	168	8	208	6.5	222	3	271	–	125	5	38.5
RCA-IM 921	36.1	6	44.0	1	77	2	113	2	157	4	213	8	252	8	251	–	113	2	33

Tab. 4. Percentage recovery of T_4 added to serum.

Added	75 nmol/l	150 nmol/l	225 nmol/l	Total
Recovered	$\bar{x}$ ± SD[1]	$\bar{x}$ ± SD[1]	$\bar{x}$ ± SD[1]	$\bar{x}$ ± SD[2]
Abbott	107.5 ± 4.7	105.8 ± 3.6	100.4 ± 2.9	104.6 ± 4.8
Becton D	109.1 ± 3.1	105.4 ± 6.6	99.9 ± 5.1	104.8 ± 6.2
Byk-Riamat	88.9 ± 6.8	85.3 ± 10.9	83.6 ± 8.5	85.9 ± 8.7
Byk-Spac T_4	98.9 ± 2.0	98.1 ± 2.2	96.2 ± 2.0	97.7 ± 2.2
Clinical Assays	101.6 ± 3.5	105.2 ± 2.8	96.0 ± 1.6	100.9 ± 4.7
Corning	107.3 ± 1.6	103.4 ± 2.7	108.2 ± 1.1	106.3 ± 2.8
DPC-Premix	111.1 ± 4.3	117.2 ± 8.5	115.2 ± 5.8	114.5 ± 6.6
Micromedic	108.5 ± 1.8	104.8 ± 5.4	107.0 ± 3.8	106.8 ± 4.0
NML	121.1 ± 2.0	107.6 ± 4.1	105.9 ± 4.1	111.5 ± 7.8
RCA-IM 801	103.8 ± 2.1	98.9 ± 1.7	97.8 ± 2.4	100.2 ± 3.3
RCA-IM 921	95.3 ± 3.6	93.3 ± 2.7	93.3 ± 1.9	94.0 ± 2.8

[1] N = 6
[2] N = 18

between the T_4-free serum and the zero control samples, except in the case of Becton D and Micromedic. For these two kits the T_4 concentration of the T_4-free serum was estimated to be less than 5 nmol/l.

For each kit the time necessary for the processing of 100 tubes was noted. With the exception of incubation times, this included all operations from writing the protocol to calculating the samples from the standard curve. Table 5 shows these times, as well as the number of samples that can be determined in an experiment using 100 tubes.

Discussion

Probably one of the best ways to show the correlation between the dose level and its response error is the precision profile (3). To construct such a graph from experimental results, however, many dose levels must be investigated. Another possibility is to divide the measuring range into two or three parts and to calculate the average response error. This can be done in several ways, none of which is to be preferred over the others. Therefore, we have decided to present all figures for the nine sera tested.

From table 2 it can be seen that there are differences in the intra- and interassay variations from kit to kit, which are difficult to explain. It has been reported that radioimmunoassays using antibody-coated tubes tend to have higher coefficients of variation (4). Our results (Becton D, SPAC T_4, Clinical Assays and Micromedic) seem to support this notion. Furthermore, the sample volume may be important. The four kits with a small presented sample volume of 10 µl (Becton D, Byk-Riamat, NML and Clinical Assays) show a rather large intra-assay variation, whereas RCA IM801 and RCA IM921 with a large sample volume (50 µl) yield comparatively good results.

From tables 3 and 4 it can be seen that certain kits yield systematically lower (Byk-Riamat) or higher (Becton D, NML) T_4 concentrations. Since there is not yet a definitive method for the measurement of T_4, it cannot be stated that the results of these kits are wrong. However, it is necessary that each laboratory determines its own normal values. Moreover, Byk Riamat combines, in comparison with other kits, a low measured level with a low percentage recovery, whereas NML shows exactly the opposite. These observations may be explained either by an incorrect description of the serum standards or by an incomplete separation of T_4 from TBG.

Concerning the labour-time per 100 tubes (tab. 5) it should be noted that all pipetting was carried out with semi-automatic pipettes. Likewise all calculations, drawing the standard curves and reading off the samples were done by hand. By automation the labour-time could be reduced. The results in this table, therefore, should be considered comparatively. As was to be expected, the kits employing an antibody-coated tube were clearly less labour-intensive.

The overall performance of any kit is determined by the way it fits a number of criteria, some of which have been investigated in this study. It is difficult to recommend or reject any kit in particular because the choice to be made depends on the demands of the investigator.

Tab. 5. Labour time for an assay of 100 tubes.

Kit	Labour time (min)	Number of samples
Abbott	160	45
Becton	115	44
Byk-Riamat	150	43
Byk-Spac T_4	115	43
Clinical Assays	115	44
Corning	155	45
DPC-Premix	160	44
Micromedic	145	43
NML	175	45
RCA-IM 801	175	46
RCA-IM 921	160	46

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